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小麦族鹅观草属三种植物的生物系统学研究*

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摘要 本文研究了禾本科小麦族鹅观草属的3个种：缘毛鹅观草（*Roegneria pendulina* Nevski $2n=4x=28$），纤毛鹅观草（*R. ciliaris* (Trin.) Nevski $2n=4x=28$）和鹅观草（*R. kamoji* Ohwi $2n=6x=42$）及其种间杂种的形态变异和染色体配对行为。各杂种F_1的减数分裂染色体配对数较高，但杂种高度不育。在杂种减数分裂中还观察到一定频率的多价体形成。以上结果充分表明该3种植物享有两个共同的基本染色体组，在S和Y染色体组之间发生过染色体相互易位，缘毛鹅观草的染色体组可拟定为S^PY^P。

关键词 生物系统学；染色体组分析；小麦族；缘毛鹅观草；纤毛鹅观草；鹅观草

BIOSYSTEMATIC STUDIES AMONG ROEGNERIA PENDULINA, R. CILIARIS AND R. KAMOJI OF THE TRIBE TRITICEAE, GRAMINEAE

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Abstract Morphological and cytological studies of three *Roegneria* species and their artificial hybrids were carried out on *R. pendulina* Nevski ($2n=4x=28$), *R. ciliaris* (Trin.) Nevski ($2n=4x=28$) and *R. kamoji* Ohwi ($2n=6x=42$). The F_1 hybrids

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showed a comparatively high chromosome pairing in meiosis, but were almost completely sterile. A few amount of multivalents were also observed in the hybrids. These results indicate that the three independent species share two same basic genomes and they are very closely related. Reciprocal translocations probably occurred between the chromosomes of S and Y genomes. The genomes of *R. pendulina* could be designated as S^pY^p.

Key words Biosystematics; Genomic analysis; *Triticeae*; *Roegneria pendulina*; *R.ciliaris*; *R. kamoji*

Introduction

Roegneria C. Koch of the tribe *Triticeae* (Gramineae) is a very large and widely distributed genus which includes about 120 species in the world and nearly 60 in China[1, 2]. Since this genus contains a large number of species, subspecies, and the morphological limits among some of them are not so clear, taxonomy seems very difficult within the genus. Furthermore, only very few cytologic and genetic studies have been carried out in the genus [3—5] thus, further biosystematic studies on different *Roegneria* species become very attractive and significant.

R. kamoji Ohwi ($2n=6x=42$) and *R. ciliaris* (Trin.) Nevski ($2n=4x=28$) are two of the species in *Roegneria* distributed most widely in China as well as in Korea and Japan. *R. penduulina* Nevski and *R.pendulina* var.*pubinodis* Keng ($2n=4x=28$) grow mainly in northeast, north and central part of China. According to Dewey and Lu's studies (Op. cit.), *R. kamoji* and *R. Ciliaris* share two basic genomes (SSYY), and they are closely related to one another. There was, however, no cytological study on *R. pendulina* reported before. In order to investigate the biosystematic relationships between *R. pendulina* and *R. kamoji*, *R. ciliaris*, three artificial hybrids were made from the crosses between *R. pendulina* and the other two species. The morphological comparison and cytological analysis of the parental species and their F_1 hybrids are reported in thepresent paper.

Materials and Methods

The species and accessions used for morphological analysis and interspecific hybridization in the present study were collected in recent years in different regions of China and are listed in Table 1. All accessions were developed from seeds.

The plants were crossed in the field of Triticeae Nursery in Guanxian, Sichuan. The hybridizations were achieved as follows: the two lowest florets of each spikelet were hand-emasculated and hte remainder was pinched off. The spike was then

enclosed in cellophane bags and pollinated two days later by brushing maternal stigmas with newly broken anthers of the paternal species. The hybrid seeds were germinated on filter paper in petri-dishes and then sowed in pots at two leaf stage.

Table 1. The materials used in the crosses

Taxa	Accession number	2 n	Location	Collecter and year
R. pendulina	86-155	28	Huaqing Pond, Xian Shaanxi	J. L. Yang&B. R. Lu 1982
R. pendulina var. *pubinodis*	86-156	28	Zhengzhou, Henan	J. L. Yang&B. R. Lu 1982
R. ciliaris	86-120	28	Erlangshan Mountain, Sichuan	J. L. Yang 1982
R. kamoji	86-143	42	Yaan, Sichang	J. L. Yang 1982

Twelve morphological characters were compared between the parents and the F_1 hybrids. For cytological studies the young spikes were fixed in Carnoy's II solution (alcohol : chloroform : acetic acid = 6 : 3 : 1), and stained with Snow's carmine at the temperature of 65°C for 48 hours. The stained anther was squashed in 45% acetic acid. Microphotographs were taken from permanent meiotic preparations. Pollen grains from mature anthers were stained in an aqueous Iodine-KI solution for pollen fertility studies.

Table 2. Results of crosses among *Roegeria* species

Combinations	No. of florets crossed	Seed set		Germination (%)	Plants obtained
		No.	%		
R. pendulina × *R. kamoji*	201	125	62.2	30.4	38
R. pendulina var. *pubinodis* × *R. kamoji*	112	95	84.8	4.2	4
R. pendulina × *R. ciliaris*	176	93	52.9	3.2	3

Table 3. Comparison of morphologial characteristics

Parents and F_1 hybrids	Height	Length of spike	Length of top internode	Length of flag leaf	No.of spikelets per spike	No.of florets per spikelets
P. pendulina	103.00 ±3.38	21.00 ±2.88	40.00 ±3.03	17.00 ±2.00	16.60 ±1.62	7.60 ±0.80
R. pendulina var. *pubinodis*	98.60 ±2.65	21.40 ±0.49	33.60 ±3.14	21.20 ±2.79	13.60 ±0.49	8.60 ±0.49
R. ciliaris	111.60 ±5.64	23.80 ±1.91	52.20 ±2.48	21.90 ±1.02	20.20 ±2.40	13.80 ±0.75
R. kamoji	146.40 ±5.08	33.10 ±1.28	45.30 ±3.29	24.90 ±1.11	26.60 ±1.20	9.80 ±0.97
R. pendulina × *R. ciliaris*	94.60 ±2.89	25.00 ±1.14	33.40 ±2.14	24.40 ±1.85	22.40 ±1.02	9.80 ±0.40
R. pendulina × *R. kamoji*	102.80 ±2.79	28.60 ±1.02	31.80 ±2.75	21.80 ±1.94	21.60 ±0.49	8.40 ±0.49
R. pendulina var. *pubinodis* × *R. kamoji*	95.00 ±4.44	24.80 ±1.47	24.60 ±3.38	20.90 ±1.89	18.40 ±1.49	8.80 ±0.40

* the length of lemma awn; ** '+' hairy, '−' hairless

Voucher specimens of the parents and the hybrids are deposited in the herbarium of Triticeae Research Institute in Guanxian, Sichuan.

Results

1. Interspecific crosses

As the results shown in Table 2, the three combinations of interspecific crosses all produced hybrid seeds and the general seed set of the combinations was rather high. The combination of *R. pendulina* (86-155) × *R. kamoji* (86-143) produced 125 well-developed seeds from 201 pollinated florets. The seeds were very easy to germinate, and developed into 38 pentaploid hybrid plants later. The combinatinos of *R. pendulina* var. *pubinodis* (86-156) × *R. kamoji* (86-143) and *R. pendulina* (86-155) × *R. ciliaris* (86-120) formed a lot of shrivelled seeds, the ratio

among the parents species and their F_1 hybrids (cm)

Length of spikelets	Length of glumes lower	Length of glumes upper	Length of lemma	Length of palea	Length of anther	Hair on lemma
4.50 ±0.13	0.86 ±0.04	0.92 ±0.04	3.26 (2.20) * ±0.08 (±0.06)	0.89 ±0.02	0.31 ±0.02	+ **
4.74 ±0.39	1.00 ±0.01	1.14 ±0.05	3.86 (2.94) ±0.34 (±0.58)	0.89 ±0.01	0.31 ±0.01	+
4.32 ±0.41	0.93 ±0.05	0.98 ±0.04	2.13 (1.70) ±0.16 (±0.14)	0.67 ±0.02	0.22 ±0.01	+
6.06 ±0.26	1.50 ±0.22	1.63 ±0.19	5.17 (3.96) ±0.26 (±0.21)	1.23 ±0.06	0.31 ±0.01	−
4.00 ±0.33	0.83 ±0.06	0.91 ±0.03	3.10 (2.20) ±0.14 (±0.10)	0.77 ±0.02	0.23 ±0.01	+
5.30 ±0.26	1.32 ±0.15	1.39 ±0.14	4.06 (3.00) ±0.27 (±0.23)	0.98 ±0.02	0.28 ±0.01	+
4.82 ±0.22	1.03 ±0.11	1.17 ±0.08	4.02 (2.92) ±0.49 (±0.47)	0.92 ±0.03	0.28 ±0.01	+

of seed set was 86.8% and 52.8%, respectively, but the seeds were difficult to germinate. Only 4 and 3 plants were obtained from the above-mentioned two combinations (Table 2). All the hybrid plants developed vigorously and set spikes later.

2. Morphological comparisons of the parental species and the hybrids

Twelve morphological characters of the parents and the hybrids were measured and are listed in Table 3. *R. kamoji* was generally larger than either *R. pendulina* or *R. ciliaris* in many aspects. There were a few characters to distinguish the three species here. The glumes, and lemmas of *R. kamoji* were glabrous, while those of *R. ciliaris* and *R. pendulina* were pubescent. *R. ciliaris* and *R. pendulina* were more similar to each other, nevertheless, the former species had more florets in each spikelet and the palea of it was shorter than its lemma, while the latter one possessed fewer florets and the palea was as long as the lemma. All hybrid plants of the

three combinations were vigorous, and their tilling, heading and flowering were normal. the general appearance of the F_1 hybrids was intermediate between their two parents or near to one of the parental species (Table 3).

3. Meiosis in the hybrids

The chromosome pairing at the MI of PMCs in the parental species and the F_1 hybrids is listed in Table 4 and the chromosome configuration are shown in Fig. 2 and 3.

Chromosome pairing of the parents in meiosis was very high with predominant ring bivalents (Table 4 and Fig. 2). Univalents were only occasionally observed in *R. kamoji* and *R. ciliaris*, and no multivalent was found. The chromosome pairing of the hybrids showed different patterns in the three combinations. The hybrids of *R. pendulina* × *R. kamoji* and *R. pendulina* var. *pubinodis* × *R. kamoji* were pentaploids ($2n=5x=35$). They had a similar meiotic pairing pattern. A large number of univalents were found in the two combinations, on the average of 11.20 and 9.88 per cell. The average number of bivalents was 11.09 (form 8—14) and 10.98 (from 6—14) per cell, respectively. A low frequency of trivalents and quadrivalents was also examined, around 0.5—0.9 multivalents cell, while the highest number of them was recorded with 3 in 6% cells (Fig. 3 F). A lot of micronuclei were observed, too, in the tetrads (Fig. 3 J). *R. pendulina* var. *pubinodis* × *R. kamoji* seemed to have higher chiasma frequency than *R. pendulina* × *R. kamoji*. As for the combination of *R. pendulina* × *R. ciliaris*, which was a tetraploid ($2n=4x=28$) hybrid, the average chromosome pairing was 3.93 univalents, 10.20 bivalents, 0.27 trivalents and 0.80 quadrivalents. The bivalents per cell variedfrom 5—14 and multivalent from 0—2. About 20% cells were observed with 2 multivalents (Table 4 and Fig. 3 G, I). All combinations had more than 17 chiasmata per cell. The highest number of chiasmata was recrded, over 21 per cell, in the combination of *R. pendulina* var. *pubinodis* × *R. kamoji* (Table 4).

4. Fertility

The pollen fertility and seed set of the parental species were normally examined and shown in Table 5. Most pollen grains of the F_1 hybrids were not viable. The pollen fertility of all combinations was very low, less than 1% in the combinations of *R. pendulina* × *R. kamoji* and *R. pendulina* var. *pubinodis* × *R. kamoji*, and less than 5% in *R. pendulina* × *R. ciliaris*. The seed set of both tetraploid and pentaploid hybrids was found extraordinarily poor, less than 0.01%, after carefully examining a great number of florets (Table 5).

Table 4. Chromosome pairing at MI of PMCs in the four parental species and their F_1 hybrids

Accession and combination		2 n	No. of cells observed	Chromosome pairing I	II Total	II Rods	II Rings	III	IV	Chiasmata per ecll
R. pendulina	86-155	28	50	–	14.00 (14)	0.26 (0—3)	13.74 (11—14)	–	–	27.74 (25—28)
R. pendulina var. *pubinodis*	86-156	28	50	–	14.00 (14)	0.44 (0—3)	13.74 (11—14)	–	–	27.56 (24—28)
R. ciliaris	86-120	28	50	0.12 (0—4)	13.88 (13—14)	0.80 (0—3)	13.08 (0—14)	–	–	27.11 (25—28)
R. kamoji	86-143	42	50	0.28 (0—2)	20.86 (20—21)	0.46 (0—2)	20.40 (19—21)	—	–	41.24 (39—42)
R. pendulina 86-155 × *R. kamoji* 86-143		35	54	11.20 (7—21)	11.09 (8—14)	5.76 (1—9)	5.31 (3—12)	0.36 (0—2)	0.16 (0—1)	17.55 (10—25)
R. pendulina var. *pubinodis* 86-156 × *R. kamoji* 86-143		35	55	9.88 (6—17)	10.98 (6—14)	3.24 (0—7)	7.76 (0—14)	0.37 (0—2)	0.51 (0—1)	21.33 (15—28)
R. pendulina 86-155 × *R. ciliaris* 86-120		28	64	3.93 (0—10)	10.20 (5—14)	4.55 (1—10)	5.56 (2—11)	0.27 (0—2)	0.08 (0—2)	18.75 (12—26)

Table 5. Pollen fertility (Pf) and seed set in the parental species and their F_1 hybrids

Parents and hybrids	No. of pollens observed	Pf		No. of florets observed	Seed set	
		No.	%		No.	%
R. pendulina	179	150	83.8	100	91	91.0
R. pendulina var. *pubinodis*	186	122	65.6	100	89	89.0
R. ciliaris	271	151	55.7	100	93	93.0
R. kamoji	135	111	82.2	100	92	92.0
R. pendulina × *R.kamoji*	400	1	0.3	3000	4	0.001
R. pendulina var. *pubinodis* × *R. kamoji*	600	1	0.2	500	0	0.0
R. pendulina × *R. ciliaris*	445	13	2.9	2000	2	0.001

Discussion

The general appearance of the *R. pendulina* × *R. kamoji and R. pendulina* var. *pubinodis* × *R.kamoji* was intermediate between parents or near to *R.kamoji* in some characters. Lemmas of all the hybrids were covered with dense hairs which were descended obviously from *R.pendulina*. Of the other 12 examined characters, 2 characters were near to *R. pendulina*, 9 of them were intermediate between two parents and only 1 was close to *R. kamoji*. The hybrids of *R. pendulina* × *R. ciliaris* were in 9 characters intermediate between two parental species and three characters exceeding the parents (Table 3). Similar results were reported by Sakamoto (1966) who compared 16 characters of the F_1 hybrids among 7 *Roegneria* species. From the morphological point of view, the authors would like to consider that the three species were very closely allied, and *R. pendulina* and *R. ciliaris* are even more closely related.

Anthers of all F_1 hybrids were quite normal in development, but the pollens were almost sterile. Very high seed sterility was the rule in the interspecific hybrids. The present data of fertility indicate that there exist very strong reproductive barriersamong the three *Roegneria* species. They could not breed and exchange genetic materials freely. They possess independent gene pools of their own. They are, therefore, three independent biological species.

The degree of chromosome pairing in an interspecific or intergeneric hybrid can be used as an index of chromosome homology as well as specific or generic evolutionary relationships〔3〕.Because chromosome pairing is a consequence of comparison along the entire length of all the chromosome, the reliability of the genome analysis method would be greater than others〔6〕. A higher amount of chromosome pairing in a hybrid indicates a comparatively close relationship between the parental species. On the contrary, lower chromosome pairing shows a more distant relationship. Therefore, the number of bivalents and multivalents in meiosis of a hybrid is one of the bases for the determination of the genome relationship between the parental species.

In the meiosis of the three different combinations, the average number of chromosome pairing was wery high.The bivalents per cell varied from 8—14, 6—14 and 5—14, respectively, all combinations had maximam of 14 bivalents which were the number of two sets of chromosomes in the tribe *Triticeae*.The chiasma frequency per cell was from 10—25, 15—28 and 12—26, also very high. This shows obviously that *R. pendluina* has two genomes which are essentially homologous to the two genomes contained in *R. kamoji* or *R. ciliaris*. According to Dewey〔7〕 and Lu et al.〔5〕, *R. kamoji* contains three genomes, SSHHYY, and *R. ciliaris* possesses two genomes, SSYY. *R. pendulina* would, therefore, be deduced to have SSYY genomes. In other words, the three *Roegneria* species share two same basic genomes, namely, SSYY. The results of cytological studies strongly supported the inference from morphological comparison. Furthermore, comparatively high frequency of multivalents presented in all the hybrids, and the highest number of them were observed to be up to three per cell. This data indicate that there are some chromosome homologies between S and Y genomes. The authors would infer that reciprocal translocations occurred between chromosomes of genome S and Y in *R. pendulina*. If it was true, the two genomes of *R. pendulina* could be slightly modified from origional SSYY genomes and, then, be designated as $S^pS^pY^pY^p$.

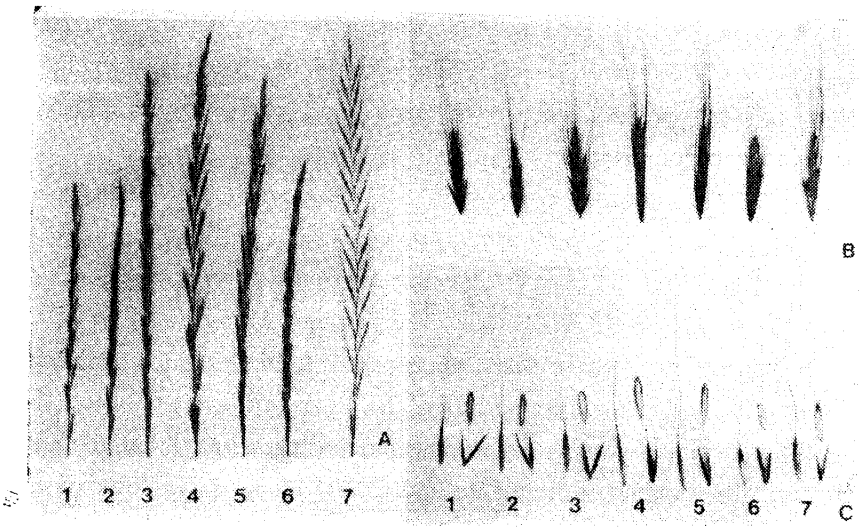

Fig. 1. A. Spikes of parental species and the hybrids.
1. *R. pendulina* (86-155); 2. *R. pendulina* var. *pubinodis* (86-156);
3. *R. pendulina* × *R. ciliaris*; 4. *R. pendulina* × *R. kamoji*;
5. *R. pendulina* var. *pubinodis* × *R. kamoji*; 6. *R. ciliaris* (86-120):
7. *R. kamoji* (86-143).
B. Spikelets, C. Glumes, lemmas and paleas of parents and the hybrids.
1. *R. pendlinua*, 3. *R.pendulina* var. *pubinodis*, 3. *R.pendulina* × *R. ciliaris*:
4. *R. pendulina* × *R. kamoji*: 5. *R. pendulina* var. *pubinodis* × *R. kamoji*:
6. *R. ciliaris*: 7. *R. kamoji*.

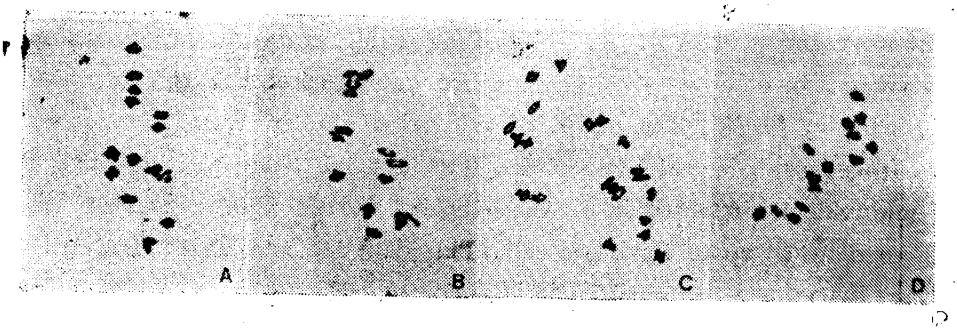

Fig. 2. Chromosome pairing at Metaphase-I of the three parental species of *Roegneria*.
A. *R. pendulina* with 14 bivalents: B. *R. pendulina* var. *pubinodis* with 14 bivalents:
C. *R. kamoji* with 21 bivalents; D. *R. ciliaris* with 14 bivalents.

Fig. 3. Chromosome pairing at metaphase-I of the F_1 hybrids.
E. *R. pendulina* × *R. kamoji* with 7 univalents and 14 bivalents; F. *R. pendulina* var. pubinodis × *R. kamoji* with 17 univalents, 2 trivalents and 1 quadrivalen; G. *R. pendulina* × *R. ciliaris* with 6 univalents, 7 bivalents and 2 quadrivalents, H. *R. pendulinaa* × *kamoji* with 10 univalents, 11 bivalents and 1 trivalent, I. *R.pendulina* × *R. ciliaris* with 4 univalents, 10 bivalents and 1 quadrivalent; J.A tetrad of *R. pendulina* × *R. kamoji* showing micronuclei in cells.

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